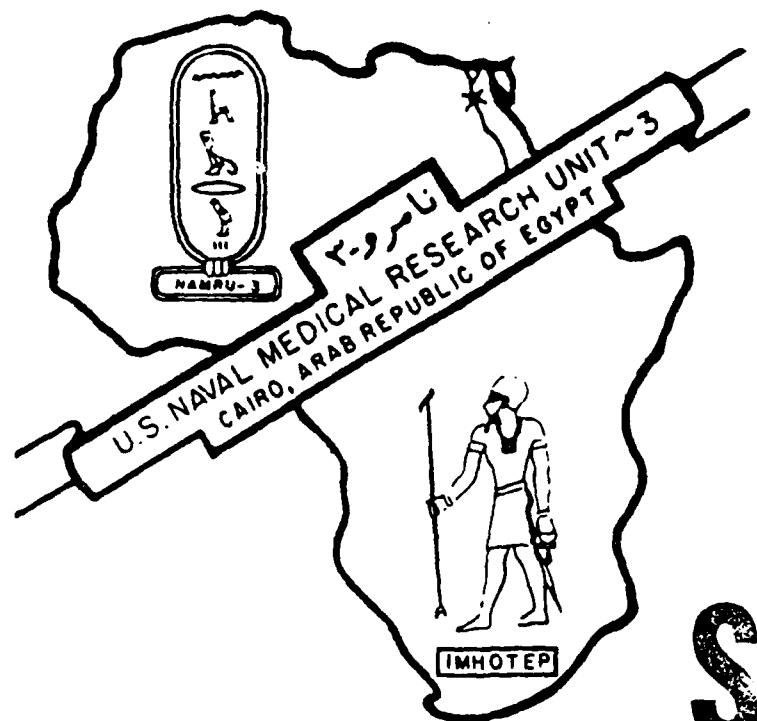


DTIC-FILE COPY

(2)

AD-A206 937



DTIC  
ELECTED  
MAR 3 1 1989  
S H D

## PUBLICATION REPORT

1518

37/88

ARBOVIRAL CAUSES OF NON-SPECIFIC FEVER AND MYALGIA IN A  
FEVER HOSPITAL PATIENT POPULATION IN CAIRO, EGYPT

BY

Medhat A. Darwish, Fred M. Feinsod, Robert McN. Scott,  
Thomas G. Ksiazek, Boulos A.M. Botros, Ibrahim H. Farrag,  
and Sherif El-Said

U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3  
(CAIRO, ARAB REPUBLIC OF EGYPT)

FPO NEW YORK 09527

DISTRIBUTION STATEMENT A

Approved for public release;

**UNCLASSIFIED**

SECURITY CLASSIFICATION OF THIS PAGE

APR 16 1987

**REPORT DOCUMENTATION PAGE**

1a. REPORT SECURITY CLASSIFICATION <b>UNCLASSIFIED</b>		1b. RESTRICTIVE MARKINGS										
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; Distribution is unlimited										
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE												
4. PERFORMING ORGANIZATION REPORT NUMBER(S)  37/88		5. MONITORING ORGANIZATION REPORT NUMBER(S)										
6a. NAME OF PERFORMING ORGANIZATION U.S. Naval Medical Research Unit No. 3	6b. OFFICE SYMBOL (If applicable) NAVAMEDRSCHU THREE	7a. NAME OF MONITORING ORGANIZATION NIH/NIAID										
6c. ADDRESS (City, State, and ZIP Code) FPO New York 09527-1600		7b. ADDRESS (City, State, and ZIP Code) Bethesda, MD										
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command	8b. OFFICE SYMBOL (If applicable) NAVAMEDRSCHDEV COM	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER NO-1 AI.2267										
8c. ADDRESS (City, State, and ZIP Code) Naval Medical Command, National Capital Region Bethesda, MD 20814-5044		10. SOURCE OF FUNDING NUMBERS <table border="1"> <tr> <td>PROGRAM ELEMENT NO. 61102A</td> <td>PROJECT NO. 3M1611- 01BS10</td> <td>TASK NO. AA</td> <td>WORK UNIT ACCESSION NO. DA301546</td> </tr> </table>		PROGRAM ELEMENT NO. 61102A	PROJECT NO. 3M1611- 01BS10	TASK NO. AA	WORK UNIT ACCESSION NO. DA301546					
PROGRAM ELEMENT NO. 61102A	PROJECT NO. 3M1611- 01BS10	TASK NO. AA	WORK UNIT ACCESSION NO. DA301546									
11. TITLE (Include Security Classification) Arboviral Causes of Non-Specific Fever and Myalgia in a Fever Hospital Patient Population in Cairo, Egypt. (UNCLASSIFIED)												
12. PERSONAL AUTHOR(S) Darwish, Medhat A.*, Feinsod, Fred M., Scott, Robert McN., Ksiazek, Thomas G., Potros, Boulos A.M., Farraq, Ibrahim H.**, and El Said, Sherif*.												
13a. TYPE OF REPORT	13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day) 1986, December, 10	15. PAGE COUNT 3									
16. SUPPLEMENTARY NOTATION Published in: Trans. R. Soc. Trop. Med. Hyg., 81:1001-1003, 1987; Acc. No. 1518.												
17. COSATI CODES <table border="1"> <tr> <th>FIELD</th> <th>GROUP</th> <th>SUB-GROUP</th> </tr> <tr> <td> </td> <td> </td> <td> </td> </tr> <tr> <td> </td> <td> </td> <td> </td> </tr> </table>		FIELD	GROUP	SUB-GROUP							18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Arboviral Infections; Non-Specific Fever.; Myalgia; Patients; Cairo.	
FIELD	GROUP	SUB-GROUP										
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Fever and myalgia are non-specific clinical manifestations of illness which commonly occur in patients with arboviral disease. In Egypt, such illness is often mis-diagnosed as "influenza". To determine arboviral aetiology in patients admitted with fever and myalgia, acute and convalescent sera samples were obtained from 55 patients admitted with these clinical manifestations to the Imbaba Fever Hospital, Giza, Egypt, during October and November 1984. Based on viral isolation, and a comparison of acute and convalescent sera, 4 patients (7%) had acute arboviral infections. Haemagglutination inhibition and indirect immunofluorescence tests showed that one had West Nile virus (WNV) infection, 2 had sandfly fever virus-Naples (SFN), and 1 had sandfly fever virus-Sicilian (SFS) infection. SFN was isolated from the acute serum sample of 1 of the 2 patients with SFN infection. Repr. n.s. (A.W.)												
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION <b>UNCLASSIFIED</b>										
22a. NAME OF RESPONSIBLE INDIVIDUAL Research Publications Branch		22b. TELEPHONE (Include Area Code) 20-2-820727	22c. OFFICE SYMBOL R.P.B.									

**UNCLASSIFIED**

## Arboviral causes of non-specific fever and myalgia in a fever hospital patient population in Cairo, Egypt

MEDHAT A. DARWISH,<sup>1</sup> FRED M. FEINSOND,<sup>2</sup> ROBERT McN. SCOTT,<sup>3</sup> THOMAS G. KSIAZEK,<sup>3</sup> BOULOS A. M. BOTROS,<sup>3</sup> IBRAHIM H. FARRAG<sup>4</sup> AND SHERIF EL SAID<sup>1</sup>

<sup>1</sup>Ain Shams University Research and Training Center on Vectors of Diseases, Cairo, Egypt; <sup>2</sup>Epidemiology and Biometry Section, MIDP, CESB, NIAID, NIH, Bethesda, Maryland, USA; <sup>3</sup>US Naval Medical Research Unit No. 3, Cairo, Egypt; <sup>4</sup>Ministry of Health, Cairo, Egypt

### Abstract

Fever and myalgia are non-specific clinical manifestations of illness which commonly occur in patients with arboviral disease. In Egypt, such illness is often mis-diagnosed as "influenza". To determine arboviral aetiology in patients admitted with fever and myalgia, acute and convalescent sera samples were obtained from 55 patients admitted with these clinical manifestations to the Imbaba Fever Hospital, Giza, Egypt, during October and November 1984. Based on viral isolation, and a comparison of acute and convalescent sera, 4 patients (7%) had acute arboviral infections. Haemagglutination inhibition and indirect immunofluorescence tests showed that one had West Nile virus (WNV) infection, 2 had sandfly fever virus-Naples (SFN), and 1 had sandfly fever virus-Sicilian (SFS) infection. SFN was isolated from the acute serum sample of 1 of the 2 patients with SFN infection.

### Introduction

Fever and myalgia are non-specific manifestations of illness. In hospitalized patients in Egypt, these symptoms are common and usually wrongly diagnosed as "influenza". Non-specific fevers with myalgia are often observed in patients with arboviral infections. Arboviruses such as West Nile virus (WNV), Sindbis virus (SIN), sandfly fever-Naples (SFN) and sandfly fever-Sicilian (SFS) viruses have been isolated from patients in Egypt (DARWISH & HOOGSTRAAL, 1981). Rift Valley fever (RVF) was epidemic in Egypt in 1977 and 1978 (IMAM & DARWISH, 1977; MEEGAN, 1979) but has not been virologically confirmed since 1980 (DARWISH & HOOGSTRAAL, 1981). Other arboviruses, such as chikungunya virus (CHIK), have been isolated in nearby African countries (BERGE, 1975).

To determine whether arboviruses might be responsible for hospital admissions in Egypt, virus isolation and serological screening were performed on sera obtained from patients hospitalized with non-specific fever and myalgia.

### Materials and Methods

#### Subjects

Patients more than 10 years of age with non-specific fever and myalgia who had been admitted to the Imbaba Fever Hospital (IFH), Giza, Egypt, were selected by the admitting physicians to be included in the study. An acute serum sample was obtained as soon as possible after onset of illness, and a convalescent sample at least 8 d following admission. Serum was separated and stored at -70°C within 2 h of collection. A standardized questionnaire, completed for each patient, included demographic information such as name, address and occupation as well as clinical manifestations of

the current illness, travel history and the prevalence of illness in the immediate family. Informed consent was obtained from all patients.

#### Viral isolation

Acute sera were inoculated on to 2 mammalian cell lines, Vero (green monkey kidney) clone E-6 and BHK (baby hamster kidney) clone-13 and one mosquito cell line, *Aedes albopictus* (C6/36) (IGARASHI, 1978). If cytopathic effect (CPE) did not develop, mammalian cell lines were blind-passaged once after 14 d. Mammalian cell cultures in which CPE was observed, and all mosquito cell cultures, were screened by the indirect fluorescent antibody test (IFAT) using a panel of flavivirus group specific polyclonal and monoclonal antibodies (HENCHAL *et al.*, 1982). Mammalian cell lines with CPE were also tested by IFAT against a panel of antisera directed against viruses known to occur in Egypt.

#### Serology

Haemagglutination inhibition (HAI) assays were performed on acute and convalescent serum samples using as antigens a panel of arthropod-borne viruses including dengue type 2, yellow fever, WNV, SIN, CHIK, and RVF virus (CLARKE & CASALS, 1958). 8 to 16 units of antigen were used at the appropriate pH. IFATs were carried out for SFS and SFN and for haemorrhagic fever viruses. Tests for antibodies to Congo Crimean haemorrhagic fever (CCHF), RVF, Ebola (Zaire and Sudan strains) (EV), Lassa fever (LF), and Marburg (MF) viruses were made using antigen slides prepared by the Center for Diseases Control, Atlanta, Georgia, USA, and for Hantaan-like virus (HLV) antibodies using antigen slides prepared by the Salk Institute (Swiftwater, Pennsylvania, USA) and provided by the US Army Research Institute for Infectious Diseases (Frederick, Maryland, USA). Sera found to contain antibodies using composite slides were examined for IgM and IgG against individual viruses (WULFF & JOHNSON, 1979). Sera containing IgM antibodies to any of the viral agents studied were assayed for rheumatoid factor (RF) by tube dilution (SINGER & PLOTZ, 1956); those with RF were excluded.

Reprint requests to: Research Publications Division, NAM-RU-3, FPO New York 09527, USA.

## Results

### *Study population*

During October and November 1985 about 45 000 patients were seen at the IFH and close to 5000 were admitted. 55 patients who conformed to the case definition were selected for virological study, including 23 females and 32 males. 18 individuals were from 10 to 20 years of age, 10 from 21 to 30 years, 14 from 31 to 40 years, 12 from 41 to 50, and one older than 50 years. All patients were from Cairo or surrounding areas.

### *Prior arboviral infections*

Acute sera from all 55 patients were screened for arboviral antibodies. 32 (58%) had HAI antibodies to WNV, 3 (6%) had HAI antibodies to CHIK, and 4 (7%) had IFA antibodies to SFS. One patient had both HAI and IFA antibodies to RVF.

### *Acute arboviral infections*

4 (7%) of the 55 patients met the criteria for acute arboviral infections. One was infected with WNV, 2 with SFN, one with SFS. The patient with WNV was a 14-year-old female who developed an 8-fold rise in HAI titre over a 9-day period. The acute sample was taken the 9th day of illness. No IgM was detected in either acute or convalescent serum samples. 2 patients had acute infections with SFN. One was a 28-year-old male with a 4-fold rise in IFA antibodies to SFN. IgM was detected at a titre of 1/32 in both acute and convalescent sera. The second was a 29-year-old male who developed both IgG and IgM antibodies 12 d after onset of symptoms. A virus, identified as SFN by neutralization tests, was isolated from the acute serum of the latter patient, obtained one d after the onset of illness. A 40-year-old female had SFS infection. She developed IgG antibodies with a titre of 1/32 within 8 d from the onset of fever. IgM was not tested. None of the patients with acute arbovirus infection had RF.

In addition to fever and myalgia, most patients, including those with acute arboviral infection, experienced other nonspecific symptoms such as weakness, malaise, chills, back pain, headache, and retro-orbital pain.

### *Discussion*

Arboviral infections were detected in only 7% of in-patients. The small number of patients and the basis of selection prevent generalization from these findings; however, the study did sample a group of patients which have perplexed local physicians concerning diagnosis and treatment. As arboviral infection usually causes mild transient fever, it may have accounted for a considerably higher proportion of out-patients. The identification of arboviral infection suggests self-limiting fevers requiring rest and hydration rather than treatment with antibiotics.

The prevalence of arboviral antibodies in the study population reflects the general experience with these infections in Egypt. WNV, for example, has been found throughout Egypt (TAYLOR *et al.*, 1956) and the prevalence of antibody found in our patient population was similar to the 50% prevalence reported by DARWISH & IBRAHIM (1975) in over 1000 Egyptian students. The absence of prior SIN infection was unexpected, but dengue was not unexpectedly absent

as only a few individuals have had antibodies to these viruses in past surveys (DARWISH & HOOGSTRAAL, 1981). The presence of CHIK antibody in 3 subjects negative for SIN was more puzzling. The usual mosquito vector, *Aedes aegypti*, has not been found in Egypt since the early 1960s (DARWISH & HOOGSTRAAL, 1981). Despite the fact that CHIK has been isolated in several countries in Africa, it has never been found in Egypt. Cross-reactivity between CHIK and other arboviruses is limited and is, therefore, unlikely. As travel abroad has become a common occurrence, we speculate that patients with CHIK antibodies may have acquired infection outside Egypt.

The presence of antibodies to SFS in 4 individuals is consistent with its common occurrence in Egypt. SFS has been isolated on several occasions from both humans (TAYLOR, 1959) and sandflies (SCHMIDT *et al.*, 1971) in Egypt, and antibodies to SFS have been common in serosurveys (DARWISH & IBRAHIM, 1975; TAYLOR, 1959). Similarly, SFN infection is also common in Egypt. In Egypt, SFN was isolated from both humans (TAYLOR, 1959) and sandflies (SCHMIDT *et al.*, 1960), and antibodies to SFN have been consistently found in serosurveys in Egypt (DARWISH & HOOGSTRAAL, 1981; TESH *et al.*, 1976). Since RVF was epidemic in Egypt in 1977-78 (IMAM & DARWISH, 1977; MEEGAN, 1979), it was not surprising to find a patient with RVF virus antibodies.

Due to the transient nature of arboviraemia, the opportunity to isolate virus from acute sera was restricted. The serum from which SFN virus was isolated was obtained one day after the onset of symptoms and contained no detectable IFA antibody to the isolated virus. Acute serum samples from the other patients were obtained more than one day after the onset of fever.

These results indicate that arboviral disease causes an "influenza-like" illness in Egyptian populations. Diagnostic investigation of such patients including serological examination of acute and convalescent sera would be appropriate from a public health aspect. Clinical and public health authorities should consider arboviral disease as an aetiology for non-specific fever and myalgia.

### *Acknowledgements*

The authors thank Dr Michael Stek, Jr, NAMRU-3, for providing administrative support and the Ministry of Health, Cairo, Egypt, and the staff at the Imbaba Fever Hospital for their important support. The work of Dr Ali Zaki and Dr Eman Abdel Maged is also appreciated. These studies were supported by NIH/NIAID Research Contract NO-1 A1.2267 and the Naval Medical Research and Development Command, NMC, NCR, Bethesda, MD 20814, Work Unit No. 3M161102BS10.AA.421 and No. 3M16270A870.AQ.126.

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy, the naval service at large or the Egyptian Ministry of Health. These data were presented in part at the Joint Meeting of the American Society of Tropical Medicine and Hygiene and the American Society of Tropical Veterinary Medicine, Miami, Florida, USA.

### *References*

Berge, T. O. (1975). *International Catalogue of Arboviruses including certain other Viruses of Vertebrates* (2nd edition).

Atlanta, Georgia, USA: US Department of Health, Education and Welfare, publication no. (CDC)-75-8301, p. 216.

Clarke, D. H. & Casals, J. (1958). Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. *American Journal of Tropical Medicine and Hygiene*, 7, 561-571.

Darwish, M. A. & Hoogstraal, H. (1981). Arboviruses infecting humans and lower animals in Egypt: a review of thirty years of research. *Journal of the Egyptian Public Health Association*, 56, 1-112.

Darwish, M. A. & Ibrahim, A. H. (1975). Prevalence of antibodies to arboviruses in Egypt. Results of a serological survey among 1,113 university students. *American Journal of Tropical Medicine and Hygiene*, 24, 981-985.

Henchal, E. A., Gentry, M. K., McCown, J. M. & Brandt, W. E. (1982). Dengue virus-specific and flavivirus group determinants identified with monoclonal antibodies by indirect immunofluorescence. *American Journal of Tropical Medicine and Hygiene*, 31, 830-833.

Igarashi, A. (1978). Isolation of a single *Aedes albopictus* cell clone sensitive to dengue and Chikungunya virus. *Journal of General Virology*, 40, 531-544.

Imam, I. Z. E. & Darwish, M. A. (1977). A preliminary report on an epidemic of Rift Valley fever (RVF) in Egypt. *Journal of the Egyptian Public Health Association*, 52, 417-418.

Meegan, J. M. (1979). Rift Valley fever epizootic in Egypt 1977-78. I. Description of the epizootic and virological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 73, 618-623.

Schmidt, J. R., Schmidt, M. L. & McWilliams, J. G. (1960). Isolation of phlebotomus fever virus from *Phlebotomus papatasii*. *American Journal of Tropical Medicine and Hygiene*, 20, 450-454.

Schmidt, J. R., Schmidt, M. L. & Said, M. I. (1971). Phlebotomus fever in Egypt. Isolation of phlebotomus fever virus from *Phlebotomus papatasii*. *American Journal of Tropical Medicine and Hygiene*, 21, 888-892.

Taylor, R. M. (1959). Phlebotomus (sandfly) fever in the Middle East. *Proceedings of the 6th International Congress of Tropical Medicine and Malaria*, 5, 149-158.

Taylor, R. M., Work, T. H., Hurlbut, H. S. & Rizk, F. (1956). Study of the ecology of West Nile virus in Egypt. *American Journal of Tropical Medicine and Hygiene*, 5, 579-620.

Tesh, R. B., Gajdamic, S. J., Rodhain, F. & Vasenjak-Hirjan, J. (1976). Serological studies on the epidemiology of sandfly fever in the Old World. *Bulletin of the World Health Organization*, 54, 663-674.

Wulff, H. & Johnson, K. M. (1979). Immunoglobulin M and G responses measured by immunofluorescence in patients with Lassa or Marburg virus infections. *Bulletin of the World Health Organization*, 57, 631-635.

Accepted for publication 10 December 1986

*Note added in proof*

After submission of this manuscript, all 55 sera collected at the IFH were tested for antibodies to *Rickettsia typhi* and *R. conorii* using IFATs. IFA slides were prepared using cells infected with certified strains of *Rickettsia* provided by Dr Greg Dash, US Navy Medical Research Institute, Bethesda, MD, USA. Acute sera from 8 patients contained antibodies against *R. typhi*. 4 of these (7.2%) were low-titre reactions and similar titres were observed in the same patients' convalescent sera, indicating prior infection. 3 patients (5.5%) exhibited the 4-fold or greater rise in titre seen in acute infections, while one (1.8%) demonstrated high fixed titres, indicating a presumptive infection. 3 of the typhus patients were male farmers, 2 aged 30 years and one aged 17 years, while one was a 30-year-old housewife. All complained of generalized symptoms including fever, malaise and headache; no rashes were reported. Among the 55 patients there was no indication of previous or current infection with *R. conorii*, although low-level cross-reactive antibodies were seen in several of the sera with antibodies against *R. typhi*. These findings increase the proportion of cases of fever and myalgia diagnosed at the IFH to 14.4% and further indicate that arthropod-borne agents may cause a significant proportion of undifferentiated fevers in Egypt.

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution:	
Availability:	
First	Second
A-1   20	